Addition of Nucleophiles to Fragment Ions in Mass Spectrometers under Electrospray-Ionization Conditions

by **Albert Horni** and **Manfred Hesse***

Organisch-chemisches Institut der Universität Zürich, Winterthurerstrasse 190, CH-8057 Zürich

Mass spectra of several aromatic compounds containing an amino or thiol group were recorded under electrospray conditions with a *Finnigan TSQ 700*, as well as on a *Bruker Esquire-LC* spectrometer. The MS/MS spectra displayed two main peaks: the first one arising from the cleavage of the amino or thiol group; the second one appeared at 18 Da higher than the first one. It could be shown that the second peak resulted from a nucleophilic addition of one water molecule to the fragment ion.

Introduction. – Electrospray ionization (ESI) is a very mild ionization method that has become one of the most important ionization techniques in mass spectrometry. A sample solution is nebulized in a high electrical field (3–6 kV) into small charged droplets. Under a stream of a drying gas (mostly N_2), the size of the droplets shrinks. When the electrical field at the surface of a droplet becomes sufficiently high, ions may be emitted from the droplet surface into the surrounding gas (ion evaporation). After this process, the ions are transported from the atmospheric-pressure source into the vacuum and the mass analyzer. The ions in the ESI source are formed in solution either from an acid-base reaction ($[M+H]^+$, $[M-1]^-$) or from a complexation with metal ions ($[M+Na]^+$, $[M+K]^+$). Because of this soft ionization, fewer or no fragments are formed in the mass spectrometer. To obtain further structural information, the tandem mass spectrometry (MS/MS) technique is applied, where a fragmentation is obtained by collision of ions accelerated by electrical forces with neutral molecules, *e.g.*, He, N_2, or Ar [1–4].

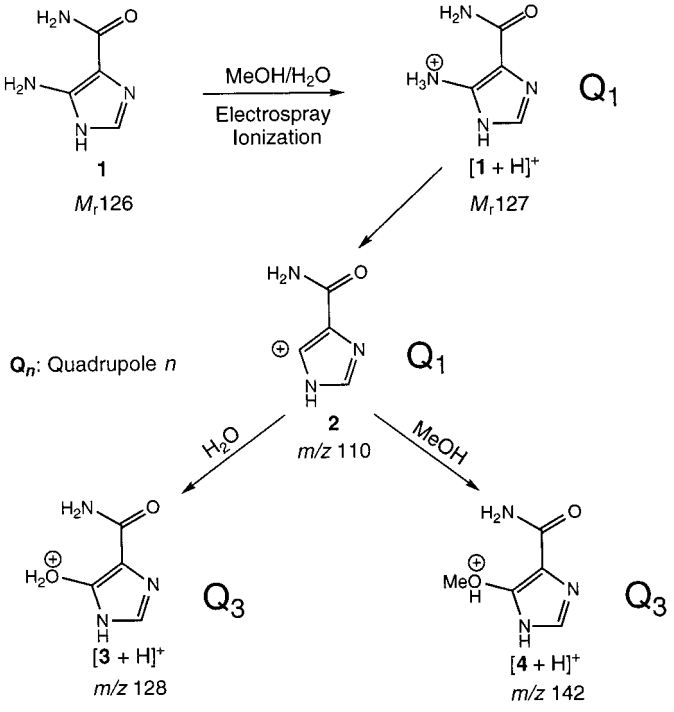
Results and Discussion. – *5-Amino-1*H-*imidazole-4-carboxamide* (**1**). The mass spectrum of the imidazole **1** with a molecular weight (M_r) of 126 was measured on a *Finnigan TSQ 700* spectrometer in a solution of MeOH/H_2O 1:1. Under these conditions, the $[M+H]^+$ signal at *m*/*z* 127 and the $[M+H-NH_3]^+$ signal at *m*/*z* 110 were detected (*Fig. 1*,*a*). After performing an MS/MS experiment on the $[M+H]^+$ ion at *m*/*z* 127 with a low collision energy (– 9 eV), an unexpected signal at *m*/*z* 128 was observed (*Fig. 1*,*b*). Under such conditions, multiple collisions between ions and neutral molecules take place. Increasing the collision energy to – 20 eV led to only two signals in the MS/MS spectrum, namely *m*/*z* 110 and 128 (*Fig. 1*,*c*). After measuring the daughter-ion spectrum of *m*/*z* 110 ($[M+H-NH_3]^+$), the only signal detected was at *m*/*z* 128. As a result of this experiment, we concluded that the parent ion of *m*/*z* 128 has to be the ion with *m*/*z* 110. On the basis of the mass difference of 18 Da, we suggest that the ion corresponding to *m*/*z* 128 should be 4-carbamoyl-1*H*-imidazole-5-hydroxonium ($[\mathbf{3}+H]^+$), obtained from a nucleophilic addition of one H_2O molecule to the ion **2**.

This reaction must take place in the vacuum chamber of the triple-state quadrupole mass spectrometer (*Fig. 2*). Even though the chamber is under vacuum (10^{-3} Torr), there are still enough solvent molecules to undergo the suggested reaction.

This reaction was also confirmed by the presence of another signal at *m/z* 142 in the ESI spectrum of **1** (*Fig. 1,a*): according to the observed nucleophilic substitution (S_N1), the ion corresponding to the signal at *m/z* 142 can be considered as the product formed from **2** (*m/z* 110) and MeOH (*Scheme 1*)[1]). Detection of this signal also in the daughter spectrum of *m/z* 127 established imidazole **1** as the origin of the ion corresponding to *m/z* 142.

Ion-molecule reactions are well known in mass spectrometry: *e.g.*, α,β-unsaturated ketones can react with nucleophiles like MeO^- under negative chemical-ionization (NCI) conditions to give the 1,2- or 1,4- addition products[2]) [5]. Dioxygen radical anions ($O_2^{-\cdot}$) can react in a flowing-afterglow (FA) apparatus with a variety of neutral organic substrates (ketones, esters, thiiranes) [6]. Under ESI conditions, nucleophilic substitution reactions of the tropylium ion ($[C_7H_7]^+$) with dimethylaminopyridine [7], and of a substituted phenylium ion with tertiary amines were observed [8]. These substitution products could be detected in a *Fourier*-transform ion-cyclotron

Scheme 1



[1]) The signal at *m/z* 149 in the ESI-MS spectrum of 5-amino-1*H*-imidazole-4-carboxamide (**1**) is the $[M+Na]^+$ adduct.

[2]) MeO^- was generated from methyl nitrite (MeONO) and MeOH. Recording the NCI spectra using only MeOH, no addition ions were obtained.

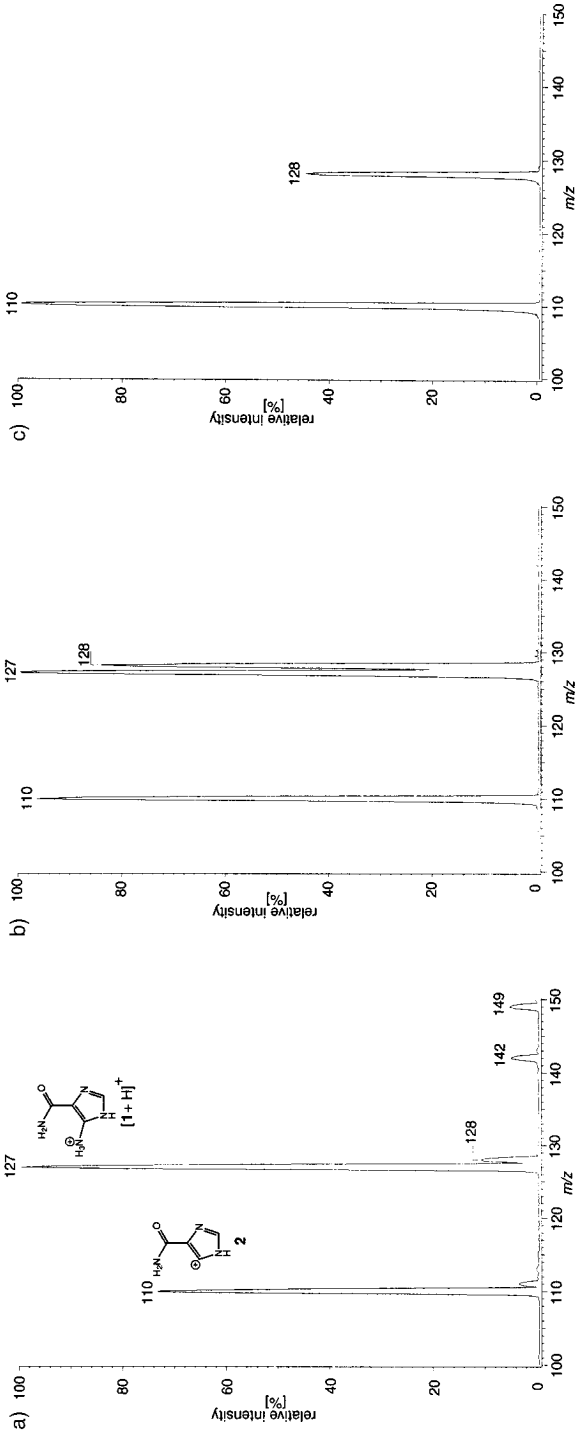


Fig. 1. *Mass spectra of 5-amino-1H-imidazole-4-carboxamide* (**1**), (M_r 126) *in MeOH/H_2O* 1:1. a) *ESI-MS Spectrum.* b) *MS/MS Spectrum at −9 eV.* c) *MS/MS at −20 eV*

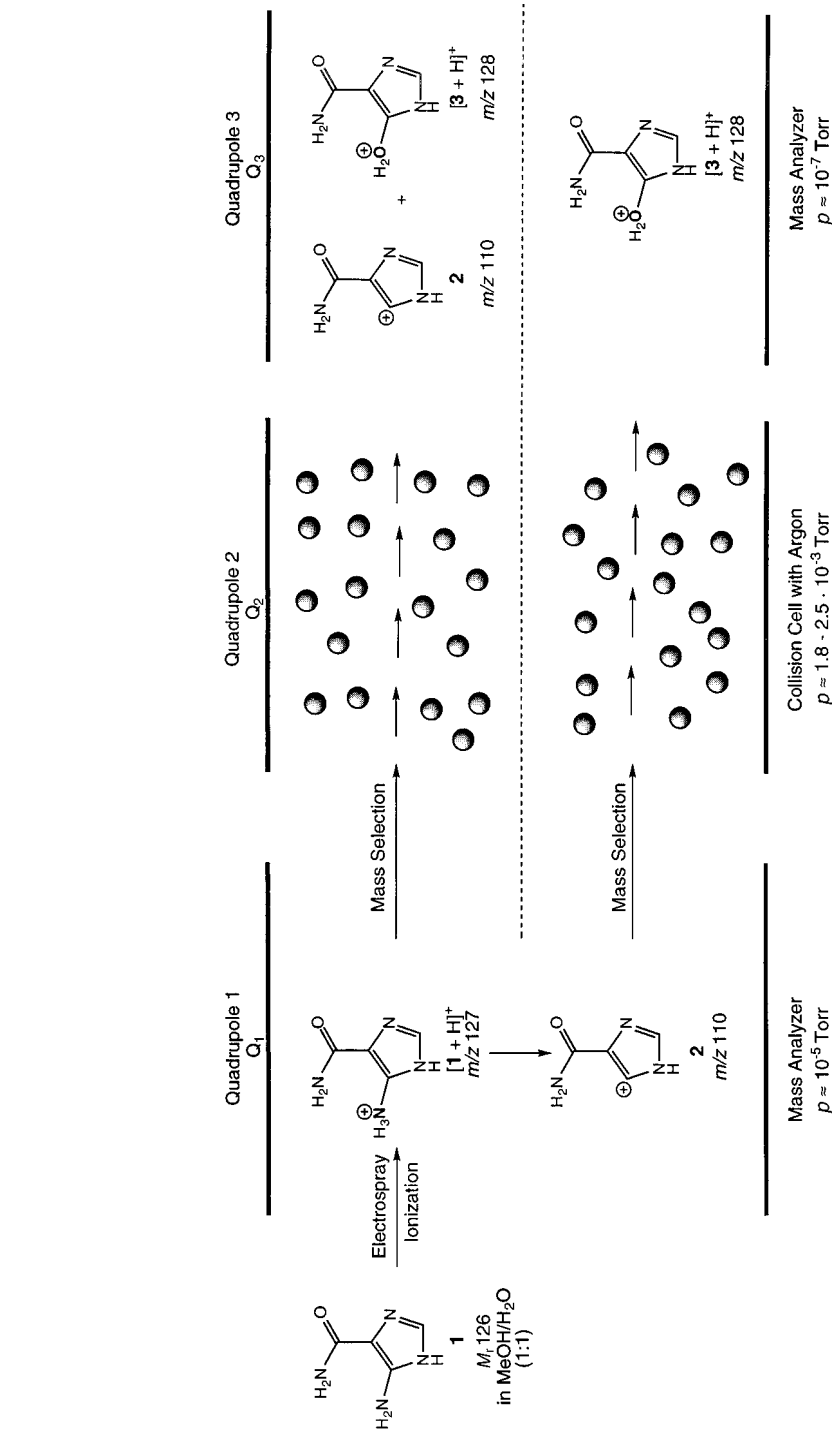
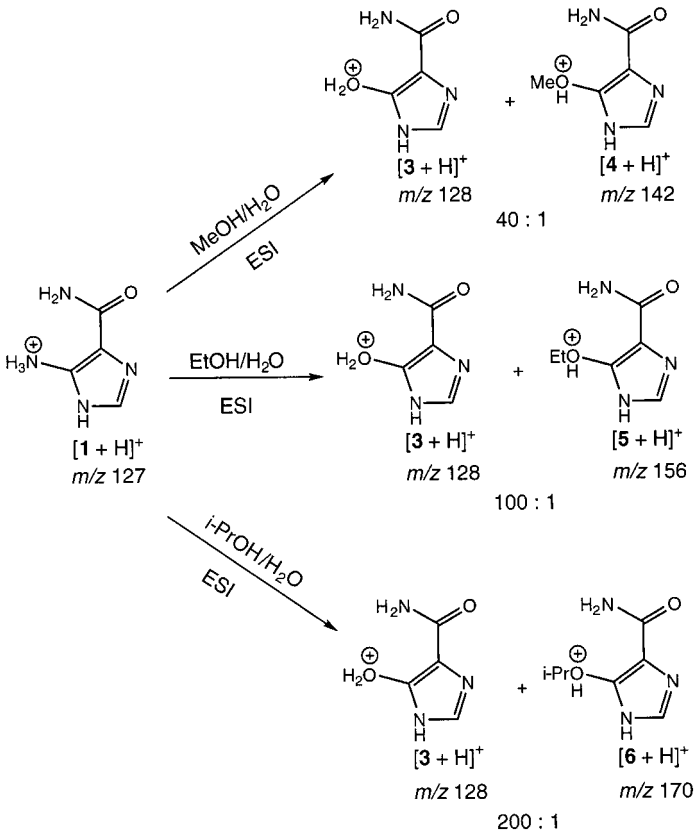


Fig. 2. *Schematic representation of a triple-state quadrupole mass spectrometer*

resonance (FTICR) mass spectrometer. However, all these experiments were carried out first by generating a charged species and then by introducing a neutral substrate *via* a leak valve to induce a reaction. In contrast to their experiments, no additional reactant was used. Our measurements were carried out without additional reactant under 'standard' ESI conditions, under which no addition reactions to fragment ions have been so far investigated nor expected.

Can nucleophiles other than H_2O and MeOH attack the aromatic carbocation **2**? To answer this question, the mass spectrum of the imidazole-4-carboxamide **1** was recorded in different solvents. In the mass spectrum of **1** in absolute EtOH, a new signal at *m*/*z* 156 appeared, which can be assigned to the product formed from reaction of the ion **2** (*m*/*z* 110) with EtOH (*Scheme 2*). Performing the MS/MS experiment of *m*/*z* 127, the signal at *m*/*z* 156 still could be observed. The same results were obtained with i-PrOH as solvent: a new signal at *m*/*z* 170 was observed, which did not disappear in the daughter-ion spectrum of *m*/*z* 127. This new ion can be assigned to the protonated i-PrO-substituted imidazole **6**. The comparison of the spectra recorded in different alcohols showed that the intensity (*I*) of the signals corresponding to the protonated alkoxy products **4**, **5**, and **6** decreased in the following order: ($I(H_2O) > I(MeOH) >$

Scheme 2



$I(\mathrm{EtOH}) > I(\text{i-PrOH})$; the following ratios have been established: $[\mathbf{3}+\mathrm{H}]^+/[\mathbf{4}+\mathrm{H}]^+$ 40 : 1, $[\mathbf{3}+\mathrm{H}]^+/[\mathbf{5}+\mathrm{H}^+]$ 100 : 1, and $[\mathbf{3}+\mathrm{H}]^+/[\mathbf{6}+\mathrm{H}]^+$ 200 : 1, corresponding to the nucleophilicity and the steric hindrance of different solvents.

The same behavior was observed for the imidazole derivative **1** in the ion-trap spectrometer *Bruker Esquire-LC*. By the ion-trap technique, MS^n experiments ($n > 2$) were performed. Isolating and fragmenting the $[M+\mathrm{H}]^+$ ion at m/z 127 led to two new signals at m/z 110 and 128 (MS^2) (*Fig. 3*). Further isolation and fragmentation of the ion

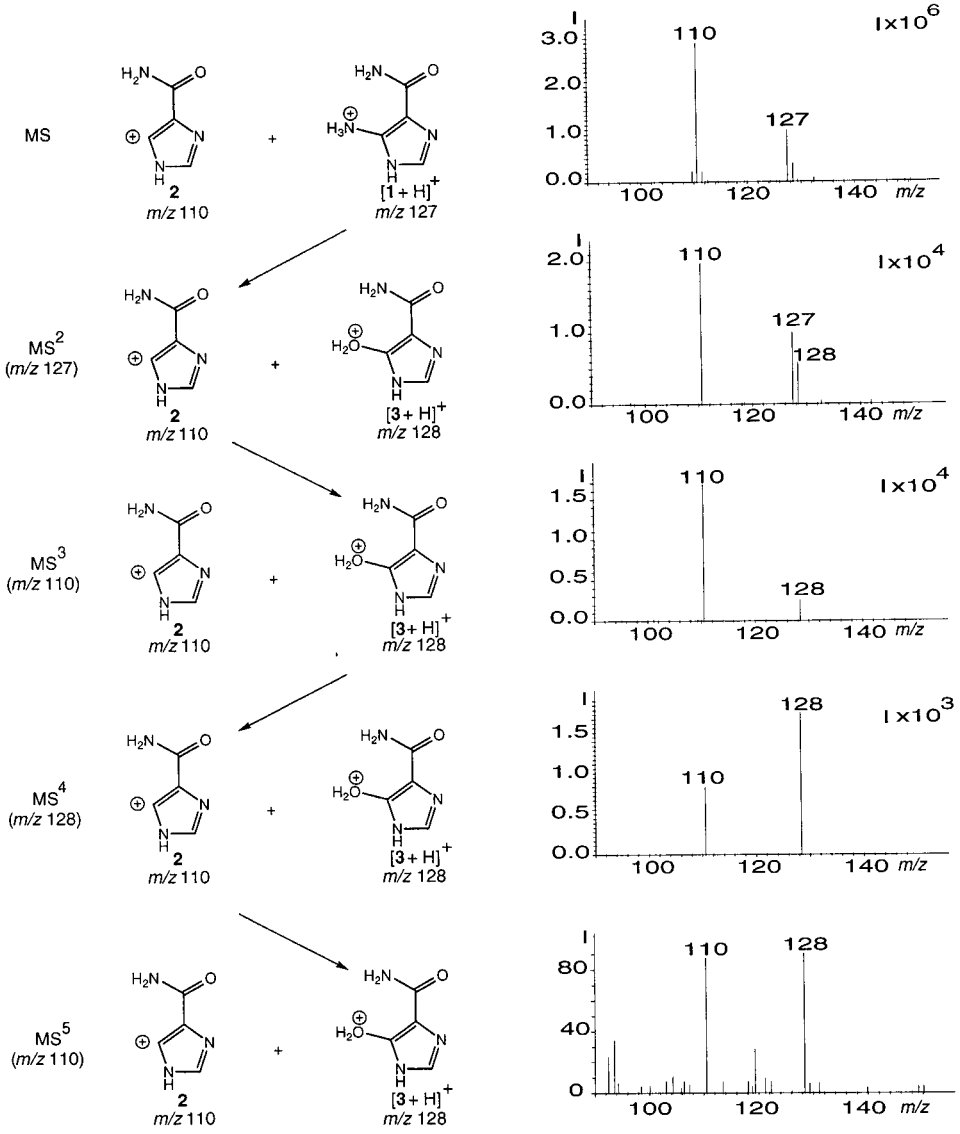


Fig. 3. *ESI-MS^n Experiments with 5-amino-1*H*-imidazole-4-carboxamide* (**1**) *in an ion-trap mass spectrometer*

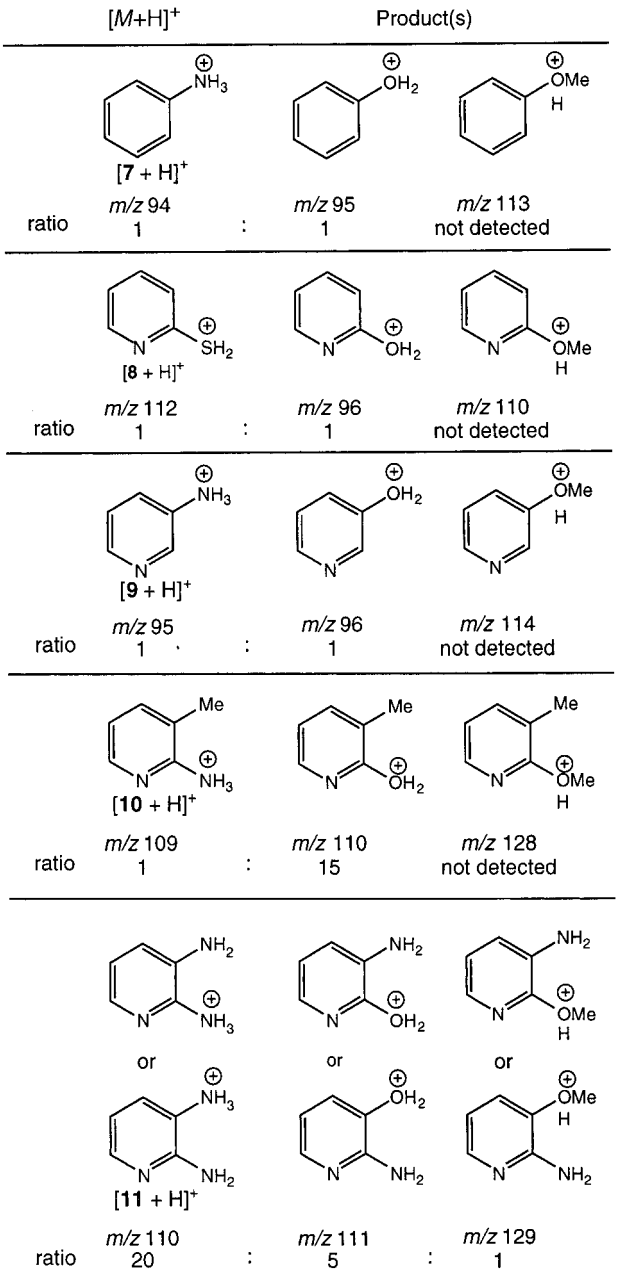


Fig. 4. *Detected products in MS/MS experiments with compounds* **7**–**11** (measured in MeOH)

at m/z 110 led to the signal at m/z 128 (MS^3). The corresponding ion was isolated, and its fragmentation resulted in a signal at m/z 110 (MS^4). Finally, an MS^5 experiment on the ion with m/z 110 was carried out to give again a signal at m/z 128. Although the

signal intensities in the last MS experiment were extremely low, it was still possible to detect the signal at *m*/*z* 128. The spectrum always showed both signals at *m*/*z* 110 and 128.

In MS/MS experiments with the following compounds, products of the reaction R–LG → R–OH (LG: leaving group) were observed: aniline (**7**), pyridine-2-thiol (**8**), pyridin-3-amine (**9**), 3-methylpyridin-2-amine (**10**), and pyridine-2,3-diamine (**11**) (*Fig. 4*). Only in the case of pyridine-2,3-diamine (**11**), addition of MeOH to the fragment ion was also detected. The MS/MS spectrum of **8** is shown in *Fig. 5*. The signal at *m*/*z* 96 corresponds to pyridine-2-hydroxonium, which was again formed from pyridinium (*m*/*z* 78) and one H_2O molecule.

No substitution reactions were detected with the following compounds: *m*-anisidine, *p*-anisidine, 2-aminobenzoic acid, 3-aminobenzoic acid, *N*-methylaniline, and *N*,*N*-dimethylaniline.

We thank the *Swiss National Science Foundation* and the *Dr. Helmut Legerlotz-Stiftung* for the financial support.

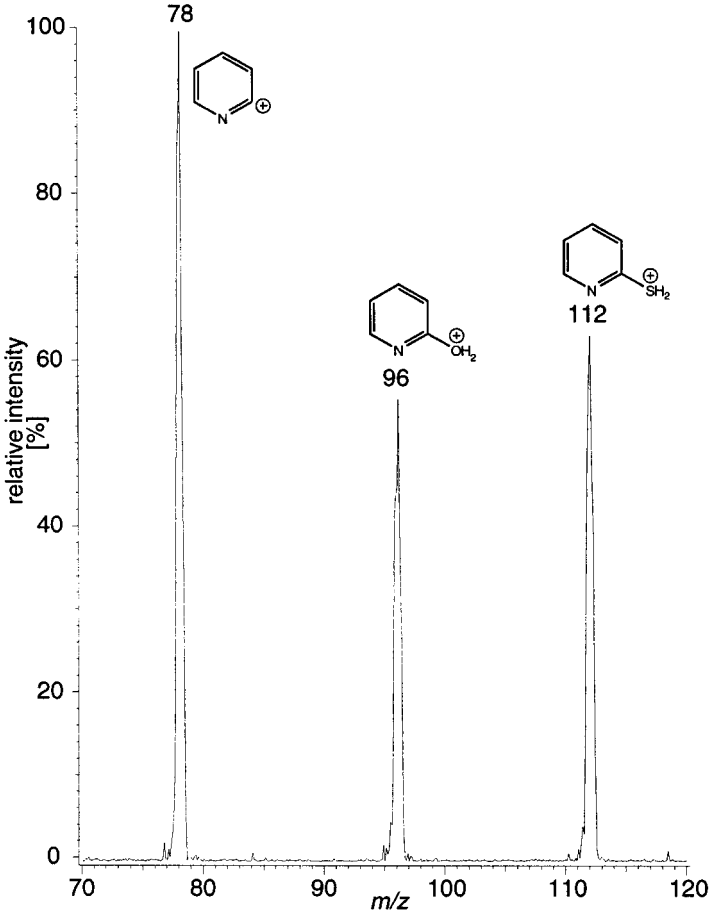


Fig. 5. *ESI-MS/MS of pyridine-2-thiol* (**8**) (M_r 111)

Experimental Part

General. All investigated compounds (obtained from *Fluka* in *purum* or *puriss.* quality) were dissolved in MeOH/H_2O 1:1, MeOH, EtOH, or i-PrOH (HPLC-grade, *Scharlau*, Barcelona, Spain) in a concentration of *ca.* 1 nmol/ml. The ESI-MS investigations were carried out with a triple-state quadrupole instrument (*Finnigan TSQ 700*, San José, CA, USA) with a combined *Finnigan* Atmospheric Pressure Ion (API) source and a quadrupole ion-trap instrument (*Esquire-LC*, *Bruker-Franzen Analytik GmbH*, Bremen, Germany) equipped with a combined *Hewlett-Packard* API source (*Hewlett-Packard Co.*, Palo Alto, CA, USA). The solns. were continuously introduced into the source with a syringe infusion pump (*Harvard Instruments*, Southnatick, MA, USA, or *Cole-Parmer Instrument Company*, Vernon Hills, IL, USA) at a flow rate of 3 µl/min.

Finnigan TSQ 700. The capillary was kept at 200°, sheath-gas pressure 30 psi; 32 spectra were averaged. For MS/MS experiments, Ar was used as collision gas with a pressure of 1.8 to 2.5 mTorr. The collision voltage was varied between −9 and −35 eV. Source-CID spectra were obtained by applying an additional voltage of − 5 to −10 eV at the octapol lens, until the source-CID spectra showed similar intensities at the same *m*/*z* values in the MS/MS spectra of the quasi-molecular ions. Source-CID fragments were further investigated in MS/MS experiments with a variation of the collision voltage between −10 and −40 eV, depending on the voltage applied at the octapole and on the quality of the obtained spectra.

Bruker Esquire-LC. Drying gas at 250° and 3.8 l/min, nebulizing gas at 21 psi, capillary voltage at 4300 V, end plate at 3800 V, capillary exit at 75 V, and *Skimmer 1* at 25 V; 8 spectra were averaged. For MS/MS spectra, the fragmentation amplitude was varied between 0.5 and 1.1 V.

REFERENCES

[1] A. P. Bruins, *J. Chromatogr. A* **1998**, *794*, 345.
[2] D. B. Hager, N. J. Dovichi, J. Klassen, P. Kebarle, *Anal. Chem.* **1994**, *66*, 3944.
[3] P. Kebarle, L. Tang, *Anal. Chem.* **1993**, *65*, 972.
[4] J. B. Fenn, M. Mann, C. K. Meng, S. F. Wong, *Mass Spectrom. Rev.* **1990**, *9*, 37.
[5] K. P. Madhusudanan, R. Jain, S. Mittal, S. Durani, R. S. Kapil, *Org. Mass Spectrom.* **1986**, *21*, 781.
[6] R. N. McDonald, A. K. Chowdhury, *J. Am. Chem. Soc.* **1985**, *107*, 4123.
[7] A. R. Katritzky, P. A. Shipkova, S. M. Allin, C. H. Watson, J. R. Eyler, *J. Mass Spectrom.* **1995**, *30*, 1581.
[8] A. R. Katritzky, R. D. Burton, P. A. Shipkova, M. Qi, C. H. Watson, J. R. Eyler, *J. Chem. Soc., Perkin Trans. 2* **1998**, 835.

Received July 16, 1999